Br. J. Pharmacol. (1989), **98**, 252–258

Kallikrein-kinin system in the plasma of the snake *Bothrops jararaca*

F.M.F. Abdalla, E. Hiraichi, [1]Z.P. Picarelli & B.C. Prezoto

Serviço de Farmacologia, Instituto Butantan Av. Dr. Vital Brazil, 1500 - Cx. Postal 65, 05504 São Paulo, SP - Brasil

1 *Bothrops jararaca* venom (BJV) caused a fall in the carotid artery blood pressure of the anaesthetized snake. This effect was tachyphylactic and was potentiated by captopril, a kininase II inhibitor; it was partially antagonized by promethazine plus cimetidine and was not affected by atropine.

2 Similar hypotensive effects were obtained by administration of trypsin or a partially purified BJV kininogenase to the snake.

3 Incubation of *Bothrops jararaca* plasma (BJP) with trypsin released a substance (or substances) that produced hypotension in the snake but not in the rat; this hypotensive effect was also potentiated by captopril.

4 The trypsinised plasma contracted *Bothrops jararaca* isolated uterus, a pharmacological preparation weakly sensitive to bradykinin. Trypsinised plasma was inactive on pigeon oviduct and rat uterus and displayed a weak action on the guinea-pig ileum. Similar effects were observed with incubates of a fraction of BJP, containing globulins, with a partially purified BJV kininogenase.

5 Like mammalian kinins, the substance(s) was(were) dialysable, thermostable in acid but not in alkaline pH, and inactivated by chymotrypsin but not by trypsin. Its(their) inactivation by BJP or BJP kininase II was inhibited by captopril.

6 These findings strongly suggest that, besides releasing histamine, BJV or trypsin release a kinin-like substance (or substances) from the snake plasma.

7 Since BJV and other kininogenases active on mammalian plasmas were shown to be unable to release kinins from BJP, in experiments conducted on pharmacological preparations suitable for the assay of mammalian kinins, these data also suggest that the snake *Bothrops jararaca*, like birds, may have developed its own kallikrein-kinin system.

Introduction

It is well known that the venom of *Bothrops jararaca* (Serpentes, Crotalinae) or trypsin decreases blood pressure of various mammalian species as a consequence of the release of histamine and bradykinin (Rocha e Silva *et al.*, 1949; Suzuki & Iwanaga, 1970). However, experiments conducted on pharmacological preparations suitable for the assay of mammalian kinins have shown that *Bothrops jararaca* venom, trypsin, and some other kininogenases, active on mammalian plasmas, were unable to release kinins from *Bothrops jararaca* plasma. After treating this plasma, in order to inhibit or destroy kininases, no evidence could be found that it contained either active factor XII (Hageman factor), prekallikrein or kininogen. Only two components of the kallikrein-kinin system were detected in *Bothrops jararaca* plasma: kininases and kininogenase inhibitors (Lavras *et al.*, 1979).

Similarly, avian plasma does not possess factor XII (Ratnoff, 1979), a factor that is involved in the activation of the blood clotting, the fibrinolytic, the C-1 complement and the kallikrein-kinin systems. However, avian plasma contains all the other components of the latter system, since a kinin is released when avian plasma is shaked with glass beads containing adsorbed mammalian or alligator factor XII (Erdös *et al.*, 1967; Seki *et al.*, 1973). In addition, Werle & Hürter (1936), Erdös *et al.* (1967), Werle & Leysath (1967) and Seki *et al.* (1973) reported that a kinin liberated from chicken plasma by pancreatic kallikrein of birds (ornitho-kallikrein) was different from mammalian kinins, in terms of its amino acid

[1] Author for correspondence.

composition and pharmacological activity. This was an indication that birds possessed their own kallikrein-kinin system. Recently, Kimura *et al.* (1987) have purified ornitho-kininogen from chicken plasma and have determined the primary structure of the kinin liberated from this substrate by bovine plasma kallikrein as being Arg-Pro-Pro-Gly-Phe-Thr-Pro-Leu-Arg. This peptide, a little different from bradykinin, induced a contraction of chicken smooth muscle and had a strong hypotensive effect in the chicken, but did not contract the rat isolated uterus.

Therefore, it was thought worthwhile to investigate the possibility of the occurrence of a kallikrein-kinin system specific for snakes not revealed by pharmacological preparations responsive to mammalian kinins. To verify such a possibility, this work started with the study of the effects of *Bothrops jararaca* venom or trypsin on the snake's own blood pressure. Further, the action of the product(s) released by incubating *Bothrops jararaca* venom or trypsin with *Bothrops jararaca* plasma was then investigated on the snake's own blood pressure and on isolated uterus.

Methods

The snakes (*Bothrops jararaca*, Serpentes, Viperidae, Crotalinae) (BJ) used in this work were collected from nature. After a quarantine of at least 15 days, they were maintained under controlled environmental conditions with a photoperiod of 12 h of light and 12 h of darkness, at a temperature of 26°C and 65% relative humidity.

Bothrops jararaca *venom* (*BJV*)

This was a pool of samples of vacuum dried venom from the Butantan Institute and contained 870 μg protein mg^{-1} of dry powder, presenting a LD_{50} equal to 2.1–2.5 μg protein g^{-1} of mouse, injected intraperitoneally. A fraction (VF) was obtained from this pool of venom, by 0.60–0.90 ammonium sulphate saturation and contained a two–three times purified kininogenase, active on rat carotid artery blood pressure.

Bothrops jararaca *plasma* (*BJP*)

Blood was collected from decapitated snakes, into polyethylene tubes containing heparin (10 iu ml^{-1} of blood) or sodium citrate (3.8 mg ml^{-1} of blood) as anticoagulant and immediately centrifuged at 8.000 r.p.m., for 30 min at 5–10°C. The resulting plasma was used fresh or after storage at −20°C.

A fraction GF, containing globulins, was obtained from this plasma by 0.0–0.5 ammonium sulphate saturation.

A purified kininase II (ACE), hydrolysing 83.3 μg bradykinin min^{-1} ml^{-1} was obtained from BJP by 0.60–0.90 ammonium sulphate saturation (Lavras *et al.*, 1980).

Pigeon plasma (*PP*)

Blood was collected from decapitated pigeons, into polyethylene tubes containing sodium citrate (3.8 mg ml^{-1} of blood). After centrifugation at 2500 r.p.m., for 15 min at 5–10°C, the plasma obtained was used fresh or after storage at −20°C.

Blood pressure recording

The carotid artery blood pressure of adult male rats (280–300 g) and of adult male or female BJ (150–600 g), anaesthetized with sodium pentobarbitone (90 mg kg^{-1}, for rats and 30 mg kg^{-1}, for snakes), i.p., was recorded on a smoked drum, with the aid of a mercury manometer. Administration of drugs was made through a polyethylene catheter introduced into the iliac vein of the rats or into the renal vein of the snakes. As the snake preparations were set up for well over 60 min, standard doses of angiotensin II (1.5 μg kg^{-1}), bradykinin (300 μg kg^{-1}) or histamine (50 μg kg^{-1}) were administered from time to time and at the end of the experiments, in order to monitor the sensitivity of the assay system.

Isolated smooth muscle preparations

Smooth muscles isolated from mammals, birds and snakes were used in the experiments *in vitro*. The rat uterus was prepared according to Gaddum & Hameed (1954) and the guinea-pig ileum as described by Henriques *et al.* (1962).

The non-atrophied oviduct of adult pigeons was removed and immediately suspended in a 10 ml chamber containing Ringer-Locke solution aerated and maintained at 42°C (Crossley *et al.*, 1980). Contractions were recorded on a smoked drum with the aid of an isotonic lever having a 6 fold magnification under a load of 0.5–1.0 g.

Pieces of the uterus (3–4 cm long) of *Bothrops jararaca* were removed from decapitated and exsanguinated snakes (150–300 g) and suspended in a 10 ml bath containing Ringer solution for snakes (Beyenback, 1984) at 30°C. Contractions were recorded under a load of 1.0–1.5 g, as described for the pigeon oviduct.

Release of active substance(s) by incubation

This was achieved by incubating either the GF fraction of BJP with VF, in the conditions described by Hamberg & Rocha e Silva (1957) or BJP with trypsin, according to Diniz & Carvalho (1963). The

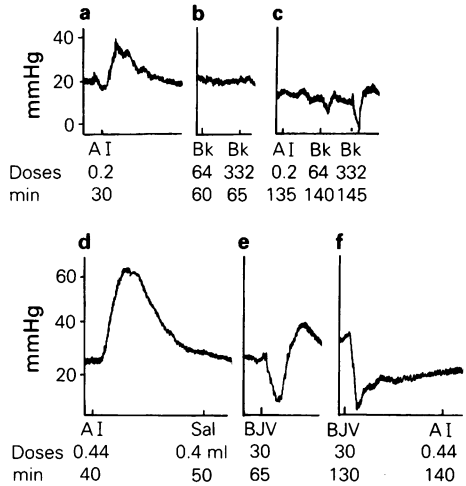

Figure 1 Action of *Bothrops jararaca* venom (BJV), bradykinin (BK) and angiotensin I (AI) on the snake's own carotid artery blood pressure. Female snakes (170 g, above and 140 g, below) were anaesthetized with pentobarbitone (30 mg kg^{-1}) i.p. and heparinized (400 iu kg^{-1}). Captopril (0.1 mg kg^{-1}) was administered at 70 min, between (b) and (c) or (e) and (f). Angiotensin II (1.5 μg kg^{-1}) produced constant effects throughout the experiment. Bk (doses in μg kg^{-1}) caused hypotension only after the administration of captopril. This drug inhibited plasma kininase II or AI-converting enzyme, since the response to AI (doses in μg kg^{-1}) disappeared following its administration. In such conditions, the hypotension caused by BJV (in mg kg^{-1}) is prolonged. Sal, 0.9% NaCl solution.

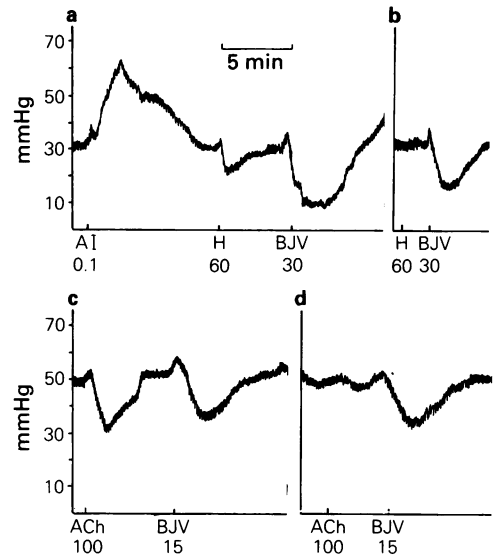

Figure 2 Effect of histamine antagonists and atropine upon the hypotension caused by *Bothrops jararaca* venom (BJV) on the same species of snake: (a) and (b) female snake (130 g); (c) and (d) male snake (120 g). Same conditions as in Figure 1. Promethazine (4 mg kg^{-1}) and cimetidine (15 mg kg^{-1}) were simultaneously administered between (a) and (b); atropine (1 mg kg^{-1} s.c. and 1 mg kg^{-1} i.v.) was injected between (c) and (d); AI – angiotensin I (doses in μg kg^{-1}). Responses to angiotensin II (1.5 μg kg^{-1}), were constant throughout the experiment.

The hypotension caused by BJV (in mg kg^{-1}) was partially blocked by the simultaneous administration of the histamine (H, in μg kg^{-1}) antagonists. Atropine did not affect the hypotension caused by the venom, in a dose that completely blocked acetylcholine (ACh, in μg kg^{-1}).

released substance(s) was(were) dried, diluted in 2 ml of 0.9% NaCl and assayed on BJ carotid artery blood pressure and rat or BJ uterus. When trypsin was incubated with BJP, the substance(s) released was(were) also tested on pigeon oviduct and guinea-pig ileum.

Pigeon plasma was incubated with trypsin according to Diniz & Carvalho (1963) and the released substance was assayed on pigeon oviduct.

Properties of the released substance(s)

The released substance(s) was(were) submitted to the following five procedures: (a) dialysis against 0.9% NaCl, at 4°C, for 24 h; (b) heating in a boiling water bath either at pH 10–11, for 5 and 10 min or at pH 1.5–2.0, for 30 and 60 min (Prado *et al.*, 1956); (c) incubation with trypsin (110 μg ml^{-1}) or chymotrypsin (37 μg ml^{-1}) (Prado *et al.*, 1956); (d) incubation at 37°C, for 15 min, with either 0.3 ml of 25 fold diluted BJP or 0.15 ml ACE and 0.2 or 0.05 ml of 0.05 M Tris-maleate buffer pH 7.4, the reaction being interrupted with boiling Ringer for snakes (1.5 or 0.8 ml respectively); (e) experiments similar to those described in (d) but BJP or ACE previously treated with captopril (170 μg ml^{-1} for BJP or 13.5 μg ml^{-1} for ACE) was used.

Drugs and reagents

The following drugs and reagents were used: sodium pentobarbitone, a gift from Abbott Laboratories of Brazil Ltd.; sodium heparin (Liquemine, Roche Laboratories, Brazil); promethazine hydrochloride (Phenergan, Rhodia Laboratories, Brazil); cimetidine (Tagamet, SKF Enila Ltd., Brazil); captopril (Capoten, SQ 14225), kindly supplied by Squibb Laboratories, Brazil; angiotensin I and bradykinin triacetate, synthesized at the Department of Biophysics, Escola Paulista de Medicina, Brazil; angiotensin II (Ciba Laboratories, Switzerland); hista-

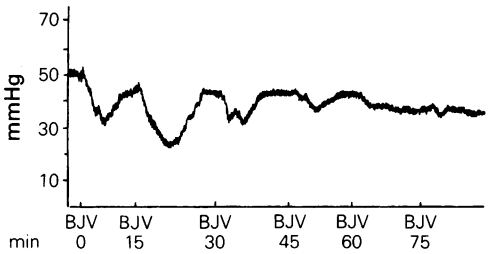

Figure 3 Tachyphylaxis to the effect of *Bothrops jararaca* venom (BJV) on the snake's blood pressure. Female snake (170 g); same conditions as in Figure 1. Responses to angiotensin II (1.5 $\mu g\,kg^{-1}$), histamine (50 $\mu g\,kg^{-1}$) and bradykinin (300 $\mu g\,kg^{-1}$), at the beginning and at the end of the experiment, were similar.

The hypotension caused by BJV diminished or even disappeared when the same dose of venom (30 $mg\,kg^{-1}$) was given repeatedly within a short interval of time.

mine free-base, acetylcholine hydrochloride, bovine pancreas trypsin (2 × crystallized, type III) and chymotrypsin (3 × crystallized, type II), (Sigma Chem. Co., U.S.A.); atropine sulphate, calcium chloride and dipotassium phosphate (E. Merck, Germany); monopotassium phosphate (J.T. Baker, U.S.A.); sodium citrate (Ecibra, Brazil).

Results

Effects of BJV, BJV kininogenase or trypsin on the snake blood pressure

As may be seen in Figure 1, BJV caused a fall in the carotid artery blood pressure of the snake, in doses as high as 10–40 $mg\,kg^{-1}$. A dose of 15 $mg\,kg^{-1}$ was equivalent to 60 $\mu g\,kg^{-1}$ of histamine or 100 $\mu g\,kg^{-1}$ of acetylcholine in producing a 20 mmHg drop in blood pressure. BJV produced a similar effect in the rat, with doses of 3–5 $mg\,kg^{-1}$. In the rat, doses of 3–6 $\mu g\,kg^{-1}$ of bradykinin produced a hypotensive effect, whereas this polypeptide showed weak effects or was even inactive on BJ blood pressure.

The hypotension caused by BJV lasted longer if the snake was treated beforehand with captopril (0.05–0.10 $mg\,kg^{-1}$), an inhibitor of angiotensin I-converting enzyme or kininase II, which in such

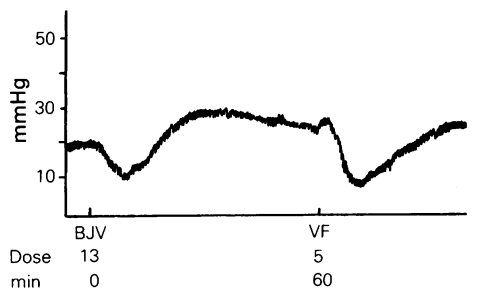

Figure 4 Effect of *Bothrops jararaca* venom (BJV) and of a partially purified venom kininogenase (VF) on the snake carotid artery blood pressure. Female snake (180 g), was anaesthetized and heparinized and treated with captopril (0.1 $mg\,kg^{-1}$), promethazine (4 $mg\,kg^{-1}$) and cimetidine (15 $mg\,kg^{-1}$). VF is a two–three times purified kininogenase obtained from BJV by 0.60–0.90 ammonium sulphate saturation. Angiotensin II (1.5 $\mu g\,kg^{-1}$) was used to check the sensitivity of the preparation. Equipotent effects were produced by BJV (in mg protein kg^{-1}) and a dose of the kininogenase almost three times smaller.

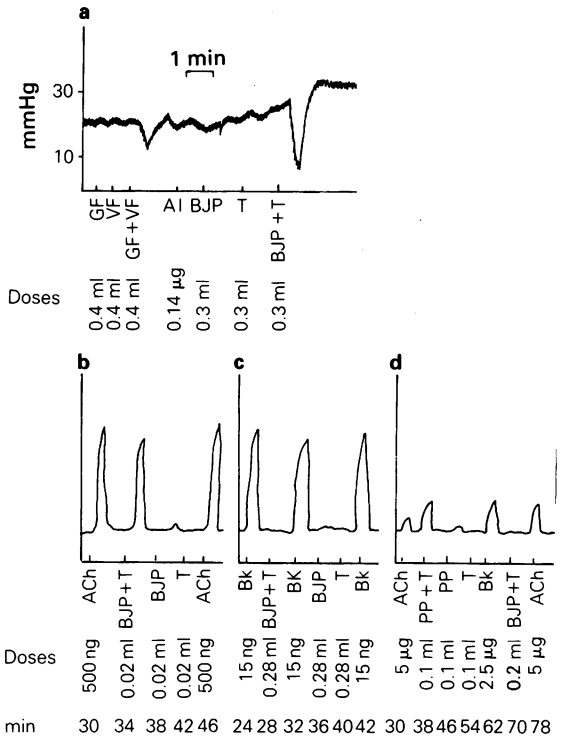

Figure 5 Effect of the substance(s) released by incubating either *Bothrops jararaca* plasma (BJP) with trypsin (T) or a fraction of BJP containing globulins (GF) with VF, a two–three times purified *Bothrops jararaca* venom kininogenase, upon the own snake's blood pressure (a) and isolated uterus (b), the rat uterus (c) and the pigeon oviduct (d). AI – angiotensin I; ACh – acetylcholine. The female snake (150 g), anaesthetized and heparinized, used for recording blood pressure, was treated beforehand with captopril (0.05 $mg\,kg^{-1}$) and gave normal responses to angiotensin II (1.5 $\mu g\,kg^{-1}$). The released substance(s), dried and diluted in 2 ml of 0.9% NaCl, was hypotensive in the snake, contracted the snake uterus but was inactive on rat uterus and pigeon oviduct. This last preparations contracted after addition of the incubate of pigeon plasma (PP) with T.

doses blocked the angiotensin I pressor response and allowed bradykinin to produce hypotension in doses that had previously been inactive (Figure 1).

After the simultaneous administration of promethazine ($4\,mg\,kg^{-1}$) and cimetidine ($15\,mg\,kg^{-1}$) to BJ, the effect of BJV was reduced. It was not modified by pretreating the snake with atropine ($2\,mg\,kg^{-1}$) (Figure 2).

As in mammals, the hypotension caused by BJV diminished or even disappeared (Figure 3) when the same dose of venom was given repeatedly to the snake within a short period of time.

Figure 4 shows that VF, a fraction of BJV containing a two–three times purified kininogenase, produced a hypotensive effect in the snake similar to that obtained with a dose of BJV two–three times greater. This effect was also potentiated by captopril ($0.05–0.10\,mg\,kg^{-1}$); it was diminished after the administration of promethazine ($4\,mg\,kg^{-1}$) plus cimetidine ($15\,mg\,kg^{-1}$) and was not modified by atropine ($2\,mg\,kg^{-1}$).

Administration of trypsin ($4\,mg\,kg^{-1}$) to BJ also caused hypotension, partially antagonized by promethazine plus cimetidine ($4\,mg\,kg^{-1} + 15\,mg\,kg^{-1}$) and not modified by atropine ($2\,mg\,kg^{-1}$).

Release of active substance(s) by incubation of GF with VF or BJP with trypsin

Incubation of GF, a fraction of BJP containing globulins, with VF, under the conditions used by Hamberg & Rocha e Silva (1957) for the liberation of kinins, resulted in the release of a substance (or substances) that caused hypotension in BJ (Figure 5). This figure also shows that a substance (or substances) with similar effect was(were) obtained by incubating BJP with trypsin, under the conditions used by Diniz & Carvalho (1963) for the determination of kininogen in various animal species. The hypotension caused by this(these) substance(s) in BJ was potentiated by a previous treatment of the snake with captopril ($0.05–0.10\,mg\,kg^{-1}$) (Figure 6). The substance(s) relased was(were) inactive on rat blood pressure.

This(these) substance(s) contracted BJ uterus (Figure 5), a preparation that does not respond to bradykinin in concentrations up to $200–300\,ng\,ml^{-1}$. The released substance(s) was(were) inactive on pigeon oviduct and rat uterus (Figure 5), but in high doses caused a weak contraction of the guinea-pig ileum. While rat uterus and guinea-pig ileum are known to react to very low doses of bradykinin, pigeon oviduct is less sensitive but contracts under the influence of the substance released by incubating pigeon plasma with trypsin.

The substance(s) released in the incubation of BJP with trypsin was(were) dialysable, showed thermostability in acid pH but not in alkaline pH. Furthermore, it(they) was(were) inactivated by chymotrypsin, BJP or ACE but not by trypsin. The inactivation by BJP or ACE was inhibited by previous incubation of BJP or ACE with captopril (Table 1).

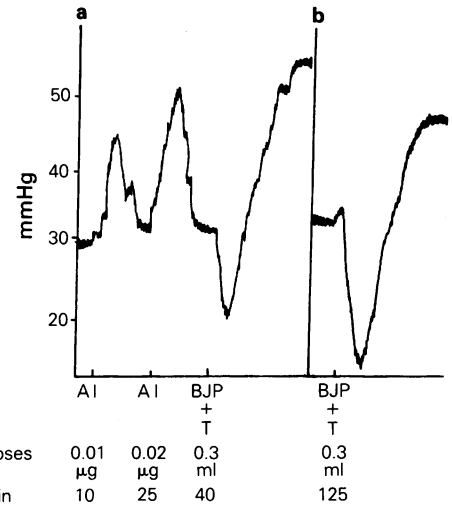

Figure 6 Effect of captopril upon the hypotension caused by the substance(s) released in the incubation of *Bothrops jararaca* plasma (BJP) with trypsin (T). Male snake (170 g) anaesthetized and heparinized; between (a) and (b), at 60 min, captopril ($0.1\,mg\,kg^{-1}$) was administered. Responses to angiotensin II ($1.5\,\mu g\,kg^{-1}$) were constant throughout the experiment. AI – angiotensin I. The hypotension produced by the released substance(s) was greater after inhibition of AI-converting enzyme by captopril.

Discussion

In mammals, small doses of venoms extracted from *Bothrops jararaca* and other snakes from the same genus mainly produce haemorrhage and hypotension (Suzuki & Iwanaga, 1970), this last effect being attributed to the release of histamine and bradykinin (Feldberg & Kellaway, 1937a,b; Rocha e Silva *et al.*, 1949) by the venoms.

In the *Bothrops jararaca* itself, BJV also caused hypotension. The decrease or even disappearance of this hypotensive effect after repeated administration of venom to the snake does not seem to be due to tachyphylaxis at receptor level since, after the disappearance of the effect, the preparation reacts to standard doses of acetylcholine, histamine or bradykinin in the same degree as before the first dose of venom. This fact suggests that, as in mammals, the venom is acting indirectly, releasing hypotensive substances

Table 1 Properties of the substance(s) released by incubating *Bothrops jararaca* plasma (BJP) with trypsin and those of bradykinin

Procedure	*Temperature* (°C)	*pH*	*Time*	*Released substance(s)*	*Bradykinin*
Dialysis against 0.9% NaCl	4	6.5	24 h	dial.	dial.
Heating	98	1.5–2.0	30–60 min	stable	stable
		10.0–11.0	5–10 min	unst.	unst.
Incubation with:					
Chymotrypsin (37 $\mu g\,ml^{-1}$)	37	6.5	5 min	inact.	inact.
Trypsin (110 $\mu g\,ml^{-1}$)	37	6.5	60 min	resist.	resist.
BJP (1:25)	37	7.4	15 min	inact.	inact.
ACE*	37	7.4	15 min	inact.	inact.
BJP (1:25) + Capt† (170 $\mu g\,ml^{-1}$)	37	7.4	15 min	resist.	resist.
ACE + Capt (13.5 $\mu g\,ml^{-1}$)	37	7.4	15 min	resist.	resist.

dial. – dialysable; unst. – unstable; inact. – inactivated; resist. – resistant.
*ACE – a purified kininase II, hydrolysing 83.3 μg bradykinin $min^{-1}\,ml^{-1}$, obtained from BJP.
†Capt – captopril (*Capoten.* SQ 14225).

and exhausing plasma substrates. Acetylcholine is not involved in this effect since it is not affected by atropine. As the duration of the hypotension decreased after the simultaneous administration of promethazine and cimetidine, H_1 and H_2 receptor blockers respectively, the release of histamine has to be considered. However, as these blockers antagonized the effect of the venom only partially, the simultaneous release of some other hypotensive substance(s) besides histamine is suggested. Taking into account the high kininase and antikininogenase activities detected in BJP (Lavras *et al.*, 1979) and the high doses of BJV necessary to produce hypotension in BJ, a kinin-like substance may be supposed to be released. It is noteworthy that high doses of bradykinin were also necessary to produce hypotension in BJ, an effect that was only achieved if the snake had previously been treated with captopril, a powerful kininase II inhibitor. The prolongation of the effect of the venom on the snake by captopril also indicates the release of a kinin-like substance.

This hypothesis is reinforced by the data showing that doses of VF two–three times smaller than those of BJV, produced similar hypotensive effects in BJ. VF contained a two–three times purified kininogenase, assayed on the rat blood pressure. In addition trypsin, a known kininogenase (Prado, 1970), caused effects in BJ, similar to those produced by BJV.

In order to confirm such a hypothesis, some *in vitro* experiments were conducted which showed that incubates of BJP or one of its fractions containing globulins, with trypsin or VF, respectively, contained a substance (or substances) that was(were) practically inactive on rat blood pressure and uterus, on pigeon oviduct and on guinea-pig ileum but that was(were) hypotensive in BJ and caused contraction of BJ uterus. Like kinins (Prado, 1970), this(these) substance(s) was(were) dialysable, thermostable at acid but not at alkaline pH, inactivated by chymotrypsin and BJ kininases but not by trypsin. Like bradykinin, the inactivation of this(these) released substance(s) by BJ kininases was inhibited by the kininase II inhibitor captopril.

All these findings strongly suggest that, as in birds (Seki *et al.*, 1973), a kinin-like substance (or substances) is(are) released in BJ by its own venom kininogenase or trypsin, a fact indicative of the presence of kininogen in the snake plasma. Furthermore, the data here presented seem to indicate that BJ has actually developed its own kallikrein-kinin system, inactive in mammals and birds and therefore, not revealed in pharmacological preparations suitable for the assay of mammalian kinins, as those used by Lavras *et al.* (1979).

The purification and characterization of the released substance(s), attempts to activate the intrinsic kallikrein-kinin system in *Bothrops jararaca* plasma and the extension of these studies to other snake species must be carried out, in order to conclude if snakes, in general, possess a specific kallikrein-kinin system.

This research was supported by Grant nos. 400324/81, 403415/82, 1020/5/83 from CNPq (Conselho National de Desenvolvimento Científico e Tecnológico, Brasil) and 84/0708-0, from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, Brasil). F.M.F.A. had a scholarship from FUNDAP (Fundação do Desenvolvimento Administrativo). We wish to thank Dr H.A. Rothschild for revising the English version, Mr Wilson D'Avila and Miss Wanda da Silva for technical assistance and Mrs Luz Marina S. de Braun for typing the manuscript.

References

BEYENBACK, K.W. (1984). Water-permeable and – impermeable barriers of snake distal tubules. *Am. J. Physiol.*, **246**, F290–F299.

CROSSLEY, J., FERRANDO, G. & EILER, H. (1980). Distribution of adrenergic receptors in the domestic fowl oviduct. *Poultry Sci.*, **59**, 2331–2335.

DINIZ, C.R. & CARVALHO, I.V. (1963). A micromethod for determination of bradykininogen under several conditions. *Ann. N.Y. Acad. Sci.*, **104**, 77–89.

ERDÖS, E.G., MIWA, I. & GRAHAM, W.J. (1967). Studies on the evolution of plasma kinins: reptilian and avian blood. *Life Sci.*, **6**, 2433–2439.

FELDBERG, W. & KELLAWAY, C.H. (1937a). The circulatory and pulmonary effects of the venom of the Australian copperhead (*Denisonia superba*). *Aust. J. Exp. Biol. Med. Sci.*, **15**, 81–95.

FELDBERG, W. & KELLAWAY, C.H. (1937b). Circulatory effects of the venom of the Indian cobra (*Naja naja*) in dogs. *Aust. J. Exp. Biol. Med. Sci.*, **15**, 441–460.

GADDUM, J.H. & HAMMED, K.A. (1954). Drugs which antagonize 5-hydroxytryptamine. *Br. J. Pharmacol. Chemother.*, **9**, 240–248.

HAMBERG, U. & ROCHA E SILVA, M. (1957). On the release of bradykinin by trypsin and snake venoms. *Arch. Int. Pharmacodyn.*, **110**, 222–238.

HENRIQUES, O.B., PICARELLI, Z.P. & OLIVEIRA, M.C.F. (1962). Partial purification of the plasma substrate for the bradykinin releasing enzyme from the venom of *Bothrops jararaca*. *Biochem. Pharmacol.*, **11**, 707–714.

KIMURA, M., SUEYOSHI, T., TAKADA, K., TANAKA, K., MORITA, T. & IWANAGA, S. (1987). Isolation and characterization of ornithokininogen. *Eur. J. Biochem.*, **168**, 493–501.

LAVRAS, A.A.C., FICHMAN, M., HIRAICHI, E., BOUCAULT, M.A., TOBO, T., SCHMUZIGER, P., NAHAS, L. & PICARELLI, Z.P. (1979). Deficiency of kallikrein-kinin system and presence of potent kininase activity in the plasma of *Bothrops jararaca* (Serpentes, Crotalinae). *Ciência e Cultura*, **31**, 168–174.

LAVRAS, A.A.C., FICHMAN, M., HIRAICHI, E., TOBO, T. & BOUCAULT, M.A. (1980). The kininases of *Bothrops jararaca* plasma. *Acta Physiol. Latinoamer.*, **30**, 269–274.

PRADO, J.L., MONIER, R., PRADO, E.S. & FROMAGEOT, C. (1956). Pharmacologically active polypeptide formed from blood globulin by a cysteine-activated protease from *Clostridium histolyticum*. *Biochim. Biophys. Acta* (Amst.), **22**, 87–95.

PRADO, J.L. (1970). Proteolytic enzymes as kininogenases. *Handb. Exp. Pharmacol.*, **XXV**, 156–192.

RATNOFF, O.D. (1966). The biology and pathology of the initial stages of blood coagulation. *Progr. Hematol.*, **15**, 204–245.

ROCHA E SILVA, M., BERALDO, W.T. & ROSENFELD, G. (1949). Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venom and by trypsin. *Am. J. Physiol.*, **156**, 261–273.

SEKI, T., MIWA, J., NAKAJIMA, T. & ERDÖS, E.G. (1973). Plasma kallikrein-kinin system in nonmammalian blood: evolutionary aspects. *Am. J. Physiol.*, **224**, 1425–1430.

SUZUKI, T. & IWANAGA, S. (1970). Snake venoms. *Handb. Exp. Pharmacol.*, **XXV**, 193–212.

WERLE, E. & HÜRTER, J. (1936). Über Ornitho-kallikrein I – Mitteilung über kreislaufaktive Substanzen der Vögel. *Biochem. Z.*, **285**, 175–191.

WERLE, E. & LEYSATH, G. (1967). Über das Molekulargewicht des plasmakinins von Vögeln. *Z. Physiol. Chem.*, **348**, 352–353.

(Received November 4, 1988
Revised February 21, 1989
Accepted April 7, 1989)